

Clinico-pathological conference

Disseminated cytomegalovirus infection

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Case presentation (Dr A Grant)

A 42 year old male Caucasian homosexual was found to be HIV antibody positive in May 1986. In June 1986 he was recruited to the MRC/ANRS Concorde Study. This is a double-blind placebo controlled study of zidovudine in asymptomatic HIV infected individuals, to assess its effect on the progression of HIV disease and survival.¹ He remained well until January 1990, when because of evidence of progression of his HIV disease (he had developed ARC symptoms with weight loss and night sweats and his CD4 count had fallen to 60/mm³ on two occasions), trial capsules were stopped and "open" zidovudine was used at a dose of 250 mg four times daily. Primary prophylaxis for *Pneumocystis carinii* pneumonia was started with monthly inhaled pentamidine. In April 1991 he was notified to CDSC as having AIDS on the basis of chronic perianal ulceration due to *Herpes simplex* infection, but otherwise remained well on zidovudine and experienced no haematological or biochemical adverse reactions to this drug. He was also taking acyclovir 200 mg as prophylaxis for *H simplex*.

In June 1991 he was admitted to hospital with a six week history of watery diarrhoea, passing up to six stools per day. He also complained of increasing right upper quadrant abdominal pain, constant in nature, made worse by ingestion of food and numbness of the feet. Examination revealed a thin man who had a low grade fever (37.4°C). The liver edge was just palpable. Fundoscopy showed evidence of cotton wool spots but no haemorrhages or other evidence of cytomegalovirus retinitis (fig 1). Investigations at this time revealed a haemoglobin of 9.7 g/dl and a white blood count of 2.1×10^9 /dl (neutrophils = 1.68×10^9 /dl). Urea and electrolytes were normal. Liver function tests showed an alkaline phosphatase of 678 (normal < 282) iu/l, AST was at the upper limit of normal at 55 iu/l, serum albumin and bilirubin levels were normal. Ultrasonography of the upper abdomen showed that the common bile duct was dilated at 7 mm; no stones were evident within the biliary tree and the pancreas was normal. A technetium IODIDA scan was performed. This is a nuclear medicine imaging technique. In normal patients following intravenous injection of the radiotracer it is taken up by the liver within five to ten minutes and is then rapidly excreted into the biliary tree, appearing in the duodenum within 20 minutes. In this patient

the scan showed rapid uptake of radiotracer into the liver parenchyma but there was delay of excretion into the duodenum and retention within the intra- and extrahepatic ducts.² These appearances were highly suggestive of sclerosing cholangitis. Stool culture revealed *Salmonella enteritidis*. Nerve conduction studies showed evidence of a mild peripheral sensory axonal type neuropathy. The patient was treated with oral ciprofloxacin 500 mg bd given for ten days for the salmonella infection.

Endoscopic retrograde cholangiopancreatography (ERCP) was performed. At ERCP the ampulla appeared inflamed and this was biopsied. A sphincterotomy was performed as the bile duct orifice appeared stenosed. A cholangiogram showed a dilated common bile duct and also dilated intra- and extrahepatic ducts.

There was little pain relief following sphincterotomy but the alkaline phosphatase enzyme levels which had risen peaking at 1208 iu/l just prior to ERCP and the AST which had risen to 98 iu/l both rapidly fell to normal levels. Histological examination of biopsy tissue revealed intranuclear and intracytoplasmic inclusions of cytomegalovirus infection (fig 2). Culture of biopsies from the ampulla later grew cytomegalovirus. Treatment with ganciclovir at a dose of 5 mg/kg twice daily was given for 21 days. He later developed a *Staphylococcus aureus* chest infection which was treated with erythromycin. A follow up stool

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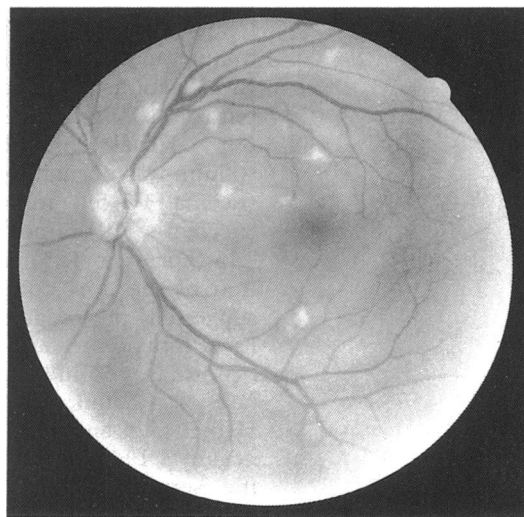


Figure 1 Fundal appearances showing numerous cotton wool spots.

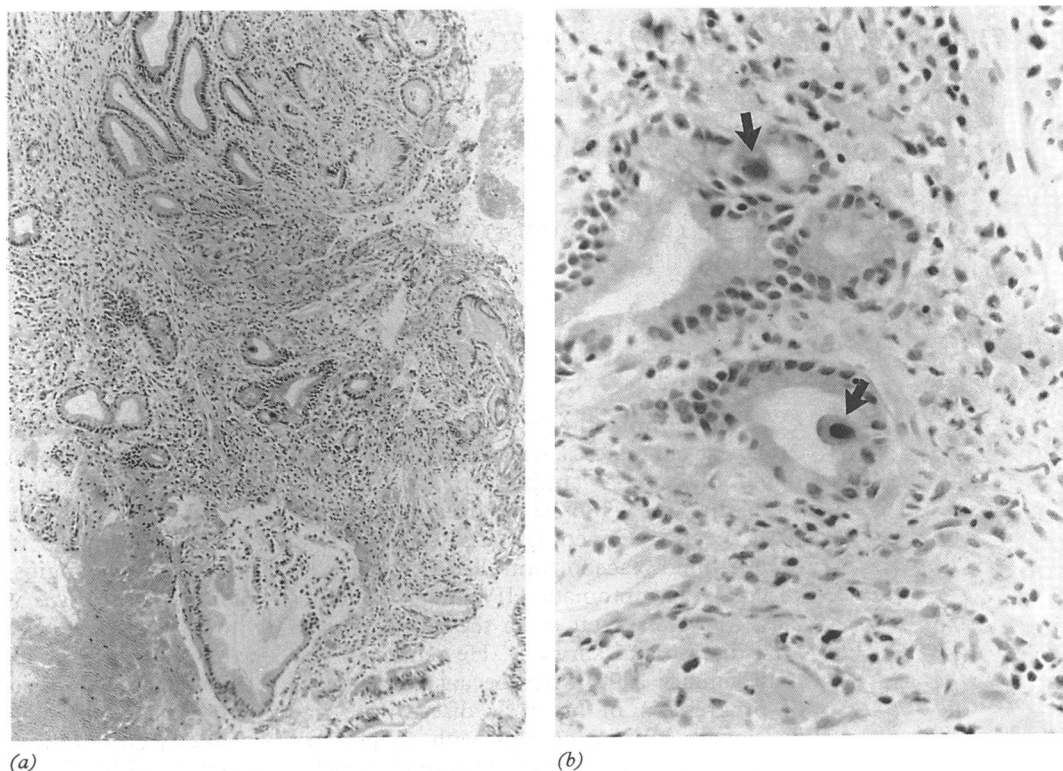


Figure 2 Ampullary biopsy obtained at ERCP (a) low power view ($\times 10$) (b) medium power view showing intracytoplasmic inclusions (arrows) $\times 40$, haematoxylin and eosin.

culture off ciprofloxacin treatment was negative for *Salmonella enteritidis*. The patient was discharged home in mid-August 1991 without diarrhoea, afebrile and well.

He was readmitted from home four weeks later. The problems were of two weeks increasing confusion and mental slowness, lethargy and weakness and unsteadiness of gait. His diarrhoea had also recurred. On examination he appeared very ill. He was pyrexial and had reduced skin turgor. He was slow to answer questions and was disorientated in time, place and person. Bilateral grasp reflexes were elicited and the reflexes were globally brisk, otherwise there were no localising signs on neurological examination. Fundoscopy was entirely normal. The cotton wool spots previously noted had spontaneously resolved. Auscultation of the chest was normal and abdominal examination revealed a 4 cm firm tender liver edge. The pulse was 114 per minute and blood pressure was 75/40 mmHg. Investigations at this stage showed a haemoglobin of 10.3 g/dl, a white blood count of 6.6×10^9 /dl (85% neutrophils). The plasma sodium was normal, serum potassium was 3.4 (normal 3.5–5.0) mmol/l and blood urea 16.7 (normal < 8.0) mmol/l. Serum creatinine was 143 (normal < 125) μ mol/l. The greater elevation of the urea compared to the creatinine suggested pre-renal failure i.e. dehydration. Liver function tests showed an AST of 174 iu/l (normal < 55 iu/l) and an alkaline phosphatase of 447 iu/l, serum albumin was 29 g/l. Hep B sAg, anti-HBc and anti-HCV were all negative. Plasma glucose was 4.2 mmol/l and the serum calcium corrected for the low albumin was normal. Multiple blood cultures were negative. An MRI scan of the head showed multiple small high signal abnor-

malities in the frontal lobe, which were non-specific. Generalised atrophic changes were noted in the cerebrum and cerebellum. These appearances were consistent with a diagnosis of HIV encephalopathy. Lumbar puncture showed clear colourless fluid. Gram, Auramine and India ink stains and subsequent culture were negative. The cryptococcal antigen was negative. No white cells were seen on microscopy and cytology revealed no abnormal cells. The CSF protein was 0.88 g/l and the CSF/blood glucose ratio was 2.1/4.7 mmol/l. Toxoplasma and syphilis serology were negative in both CSF and blood.

It was felt that the patient was dehydrated secondary to septicaemia. He was treated with intravenous cefuroxime and gentamicin. A central line was placed; the central venous pressure was +1 (normal 5–8 cm) H₂O. With fluid resuscitation the blood pressure rose to 95/55 mmHg and the temperature fell to normal over the next ten hours. Subsequently there was a further rapid deterioration in the patient's clinical condition. He became hypotensive with a tachycardia and his fever recurred. Re-examination of the chest at this point revealed end inspiratory crackles at both bases. Arterial blood gases taken with the patient breathing room air revealed pH = 7.45, PO₂ = 8.0 kPa and PCO₂ = 3.8 kPa. The plasma bicarbonate was 20 mmol/l and the standard bicarbonate was 24 mmol/l (normal). A chest radiograph taken at this point (fig 3) showed bilateral interstitial shadowing in the mid and lower zones, sparing the apices and costophrenic angles. A clinical diagnosis of *Pneumocystis carinii* pneumonia was made and treatment with intravenous high dose cotrimoxazole was begun; adjunctive therapy with methylprednisolone 1 g intravenously on

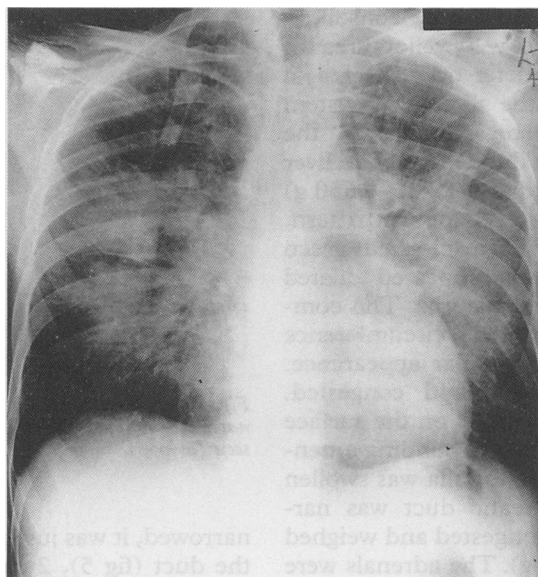


Figure 3 Chest radiograph showing bilateral interstitial shadowing in the mid and lower zones.

three successive days was commenced. Supplemental oxygen was given but despite this the PO_2 fell to 7.7 kPa with the patient breathing 60% oxygen via a face mask. Despite institution of continuous positive airways pressure ventilation the patient continued to deteriorate and he was transferred to the intensive care unit for mechanical ventilation. A metabolic acidosis then developed. This occurred on the background of a normal blood glucose, negative urinary ketones and normal urea and electrolytes and corrected serum calcium. The serum amylase was 400 (normal < 280) iu/l. The patient became increasingly acidotic and hypoxaemic and subsequently died.

Discussion (Professor I V D Weller)

The patient was admitted with a progressive six week history of watery diarrhoea and right upper quadrant abdominal pain and I note whilst he was taking zidovudine haematological and biochemical monitoring had remained normal. The clinical presentation together with the cholestatic liver function abnormalities are highly suggestive of sclerosing cholangitis and you confirmed this diagnosis both with the technetium IODIDA scan and at ERCP. At this stage two thoughts go through my mind. In HIV positive patients sclerosing cholangitis is often associated either with the presence of cytomegalovirus or cryptosporidium.³ Indeed, my initial thought was that you would find cryptosporidium in the stool. Instead, *Salmonella enteritidis*⁴ was identified and cytomegalovirus infection in the ampulla was demonstrated histologically and on culture. Following the sphincterotomy and coinciding with ciprofloxacin treatment the liver function tests returned to normal. It is not clear whether this improvement was due to the sphincterotomy or to treatment with antibiotics; this biochemical improvement occurred before ganciclovir treatment was given. Other organisms that have been found in

association with sclerosing cholangitis in HIV positive patients include Gram negative bacteria.^{3,5} I just wonder whether he also had *Salmonella enteritidis* in his biliary tree?

Following recovery from this episode he was at home for a period of several weeks and then re-presented in a critically ill condition, hypotensive, pyrexial, confused, dehydrated and subsequently developed hypoxia and diffuse shadowing on the chest radiograph. This presentation was clearly compatible with a septicaemia but blood cultures were negative. The development of an abnormal chest radiograph and hypoxaemia then suggested he had a pulmonary problem. The chest radiograph and hypoxaemia are entirely compatible with *Pneumocystis carinii* pneumonia. Another possibility was that he had a cytomegalovirus pneumonitis? You had already isolated cytomegalovirus from one site and although you had treated him for three weeks with full dose ganciclovir this was not followed by maintenance therapy. However, it is rare to find cytomegalovirus causing a diffuse pneumonitis in HIV positive patients as a single pathogen. It usually occurs as a co-pathogen and we know that by treating the co-pathogen we can usually secure recovery without the need to specifically treat cytomegalovirus.⁶ So my guess is that if there was cytomegalovirus in this man's lungs then there was also another infection as well, either *Pneumocystis carinii* pneumonia or a bacterial infection.

MRI of the head showed cortical and cerebellar atrophy and non-specific areas of high signal. These appearances are well described in HIV infection and are compatible with a diagnosis of HIV encephalopathy. Despite general support with of broad spectrum antibiotics and antipneumocystis treatment with adjunctive steroids the patient's condition deteriorated and he died. As the story unfolded I began to lean increasingly towards a diagnosis of disseminated cytomegalovirus infection. This man had advanced HIV disease and his CD4 count had been recorded at 60/mm³ 20 months before his terminal admission.

Clinical diagnoses

- 1 In the lung *Pneumocystis carinii* pneumonia? or bacterial pneumonia with evidence of cytomegalovirus infection.
- 2 In the abdomen sclerosing cholangitis with evidence of cytomegalovirus in the biliary tree, gut and liver, adrenals and I just wonder about the pancreas.
- 3 HIV encephalopathy and possible cytomegalovirus infection.

Pathology (Dr C Sargent and Professor L Michaels)

The body was that of a thin, Caucasian man. There was no evidence of Kaposi's sarcoma on the skin or palate. Microscopic examination of the respiratory system showed that the larynx, trachea and bronchi were congested and the airways contained pink fluid. The right lung weighed 1,900 g (normal = 500–600 g), the left lung weighed 1,750 g (normal = 450–

500 g). All lobes showed rubbery consolidation and at the apices there were yellow areas of softening 0.5 cm in diameter over an area of approximately 3 cm. In the alimentary system no mucosal lesions were detected in the oesophagus, stomach or duodenum. The liver weighed 2,520 g (normal = 1,400–1,650 g) and was pale with an enhanced lobular pattern. The bile ducts contained inspissated green material. The gall bladder appeared dilated and also contained green material. The common bile duct was dilated, circumference 2.2 cm, the wall had a granular appearance. The pancreas was swollen and congested. There was possible fat necrosis on the surface of the pancreas and on the surrounding omental tissue. The pancreatic ampulla was swollen and the proximal pancreatic duct was narrowed. The spleen was congested and weighed 480 g (normal 125–195 g). The adrenals were swollen and adherent to the surrounding adipose tissue.

Histological examination showed the lung parenchyma to be very abnormal, with changes being most marked in the apices. The overall pattern was that of diffuse alveolar damage with intra-alveolar haemorrhage. *Pneumocystis carinii* pneumonia with atypical histological appearance was present.⁷ There was widespread intra-alveolar foamy exudate within which organisms of *Pneumocystis carinii* were seen. There was also sub-pleural emphysematous change with bulla formation, interstitial inflammation and fibrosis with patchy calcification (fig 4). *Pneumocystis carinii* organisms were seen within the areas of calcification and there was a focal granulomatous response in association with this. In addition, throughout both lungs there was widespread evidence of cytomegalovirus infection with infected cells containing both intranuclear and intracytoplasmic inclusions. The viral infection was sufficiently severe to have contributed to the interstitial inflammation and alveolar damage.

Dissection of the pancreatic and biliary trees revealed a normal anatomical variation, seen in 5% of the population, with the common bile duct and pancreatic ducts opening separately onto the duodenal papilla rather than at a common orifice as is more usually the case. As noted at autopsy the ampulla was oedematous. The orifice of the common bile duct was

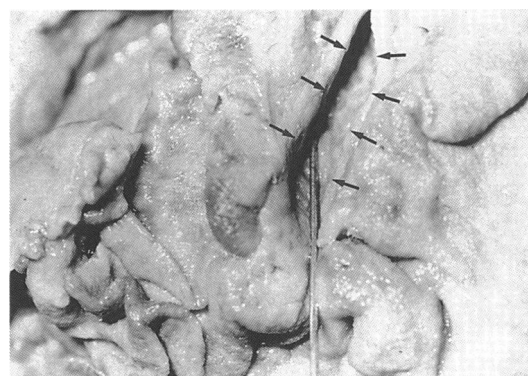


Figure 5 Close up showing a pin placed through the narrowed ampulla. The dilated common bile duct is clearly seen (arrows).

narrowed, it was just possible to pass a pin into the duct (fig 5), 2 cm proximal to the orifice the duct was dilated. The pancreatic duct was similarly narrowed. Histological examination of the duodenal papilla adjacent to the ducts showed oedema, a mild chronic inflammatory cell infiltrate and widespread evidence of cytomegalovirus infection (fig 6). The main pancreatic duct and its tributaries showed striking squamous metaplasia with areas of occlusion of ducts by metaplastic epithelium and damming up of inspissated secretions. The periductal tissue showed fibrosis and large numbers of cells with cytomegalic inclusions. The pancreatic parenchyma showed mild acinar atrophy, fibrosis and patchy autolysis but no fat necrosis. Within a pancreatic vessel collections of *Pneumocystis carinii* organisms were noted.

The wall of the common bile duct was oedematous and fibrosed with evidence of cytomegalovirus infection throughout its length. Cytomegalovirus inclusions were also seen in the gallbladder which was thick walled, oedematous and chronically inflamed. At the porta hepatis the large bile ducts contained inspissated bile and in the surrounding connective tissue there were many cells with viral inclusions (fig 7). The liver was congested but the parenchyma was largely normal. Occasional cells lining sinusoids contained cytomegalovirus inclusions (fig 8). The spleen was congested and occasional cells contained viral inclusions. Both adrenal glands showed a necrotising cytomegalovirus adrenalitis.

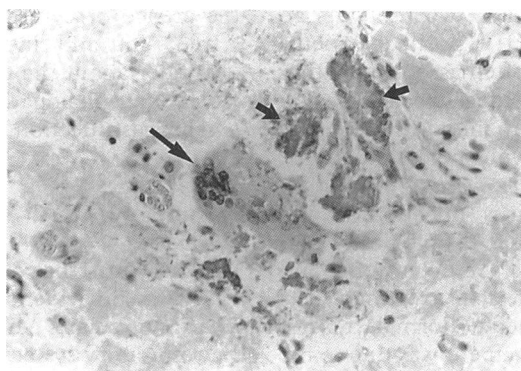


Figure 4 Section through lung apex showing eosinophilic exudate, within which are organisms of *Pneumocystis carinii* (long arrow), in addition there is microcalcification (short arrows) $\times 40$, haematoxylin and eosin.

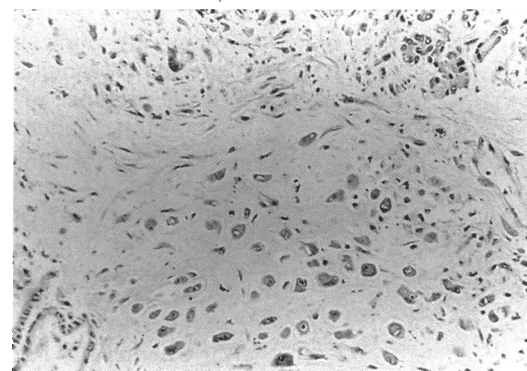


Figure 6 Head of pancreas adjacent to main pancreatic duct showing multiple cytomegalovirus inclusions $\times 25$, haematoxylin and eosin.

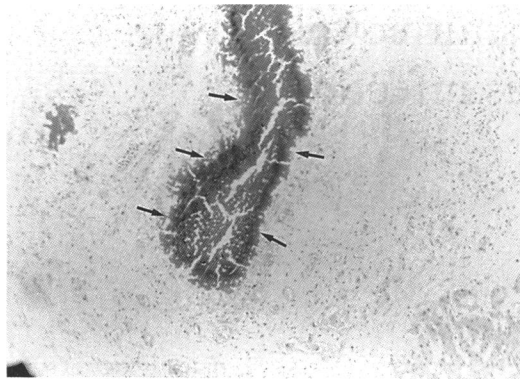


Figure 7 Bile ducts at porta hepatis showing inspissated bile (arrows) × 10 haematoxylin and eosin.

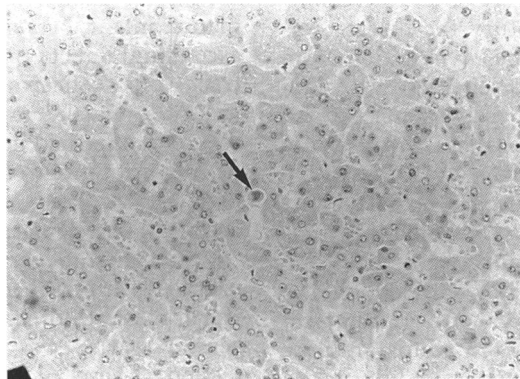


Figure 8 Liver sinusoid showing cytomegalovirus inclusion (arrow) × 25 haematoxylin and eosin.

Neuropathology (Dr F Scaravilli)

The brain, which weighed 1560 g, was symmetrical and of normal size. The leptomeninges were thin and transparent. Coronal sections showed normal appearances. No macroscopic abnormalities were seen in the cerebellum, brain stem and spinal cord.

Histological examination was carried out using routine methods. Immunohistochemical techniques were applied using the following antibodies: anti-GFAP, HAM56 for microglia, p24 for HIV and anti-cytomegalovirus. The cortex showed a discrete diffuse increase in the number of GFAP positive glial cells and contained numerous microglial nodules. These were usually of small size and consisted of rod cells and smaller numbers of macrophages and included an occasional large astrocyte containing an intranuclear inclusion which were immunostained by the CMV antibody. No multinucleated giant cells were seen and immunostaining with p24 was negative. The spinal cord showed myelin pallor circumscribed to the gracile tract.

In conclusion, the appearances were those of cytomegalovirus encephalitis, no HIV encephalitis was evident. In addition there was pallor of the gracile tract.

Pathological diagnosis

- 1 Disseminated cytomegalovirus infection
 - lung
 - pancreas
 - biliary tree
 - adrenals
 - spleen
 - brain

2 Disseminated *Pneumocystis carinii* infection with atypical pulmonary features

- bullae formation
- diffuse alveolar damage
- interstitial fibrosis
- calcification
- granuloma formation
- intra-alveolar haemorrhage

Discussion (Dr R F Miller)

Presumably because this patient had received inhaled pentamidine and not systemic prophylactic therapy for *Pneumocystis carinii* the lung abnormalities were most evident in the apices. We know that predominantly apical disease can occur in this patient group.⁸ The lack of systemic prophylaxis may also account for the finding of *Pneumocystis carinii* in the pancreas, ie disseminated *Pneumocystis carinii* infection.⁹ The terminal event in this man looks to likely to have been an Addisonian crisis.

Professor IVD Weller

I think we are going to see even more patients like this in the future as the nature of HIV disease is modified. Effective treatment and prophylaxis for many of the opportunistic infections will improve. We are already seeing an improved survival of patients with established CDC group IV disease. Increasingly the causes of morbidity and mortality will be the opportunistic malignancies, neurological disease and also infections where we have little in the way of really effective therapy, such as disseminated *Mycobacterium avium intracellulare*, cytomegalovirus infection and progressive multifocal leucoencephalopathy. With respect to the disseminated cytomegalovirus infection, in 1989 progressive cytomegalovirus disease associated with ganciclovir resistant viruses was reported.¹⁰ The mechanism of this resistance is still not clear. This patient had only received 21 days of ganciclovir therapy but it would be interesting to know whether his agonal cytomegalovirus isolates were sensitive to the drug.

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